

B

55
--53--

WHAT IS CLAIMED IS:

1 1. A resin-protein/peptide complex which comprises a resin and a target
2 protein or peptide bound thereto wherein said resin comprises
3 a) a solid support matrix; and
4 b) selected ionizable ligand covalently attached to the matrix
5 wherein the ionizable ligand is selected such that the resin is
6 electrostatically uncharged at the pH where the target protein or peptide is
7 bound to the resin and is electrostatically charged at the pH where the target
8 protein or peptide is desorbed from the resin and further wherein about 50
9 percent or more of the target protein or peptide in an aqueous medium binds to
10 the resin when the aqueous medium has either a high or a low ionic strength.

1 2. The resin-protein/peptide complex of Claim 1 wherein the ionizable
2 ligand is electrostatically uncharged at the pH where the target protein or
3 peptide is bound to the resin and is positively charged at the pH where the
4 target protein or peptide is desorbed from the resin.

1 3. The resin-protein/peptide complex of Claim 1 wherein the ionizable
2 ligand is electrostatically uncharged at the pH where the target protein or
3 peptide is bound to the resin and is negatively charged at the pH where the
4 target protein or peptide is desorbed from the resin.

1 4. The resin-protein/peptide complex of Claim 1 wherein the ionizable
2 ligand comprises an ionizable functional group directly attached to the solid
3 support matrix.

1 5. The resin-protein/peptide complex of Claim 1 wherein the ionizable
2 ligand comprises a spacer arm and at least one ionizable functionality wherein
3 the ionizable functionality is attached to the solid support matrix via the spacer
4 arm.

=

B.

56
-- 34 --

1 6. The resin-protein/peptide complex of Claim 1 wherein the solid
2 support matrix is functionalized with carboxyl groups which are protonated at
3 the pH where the target protein or peptide is bound to the resin and are
4 deprotonated and negatively charged at the pH where the target protein or
5 peptide is desorbed from the resin.

1 7. The resin-protein/peptide complex of Claim 1 wherein the resin
2 further comprises non-ionizable ligands.

1 8. The resin-protein/peptide complex of Claim 7 wherein the percentage
2 of non-ionizable ligands attached to the solid support matrix based on the total
3 of ionizable and non-ionizable ligands ranges from greater than 0% to about
4 80%.

1 9. The resin-protein/peptide complex of Claim 8 wherein the percentage
2 of non-ionizable ligands attached to the solid support matrix based on the total
3 of ionizable and non-ionizable ligands ranges from greater than 0% to about
4 40%.

1 10. The resin-protein/peptide complex of Claim 1 wherein the solid
2 support matrix is cross-linked.

1 11. The resin-protein/peptide complex of Claim 1 wherein the resin
2 contains from about 0.05 mmol to about 0.5 mmol ionizable ligand per ml of
3 the solid support matrix prior to covalent attachment of any non-ionizable
4 ligand.

1 12. The resin-protein/peptide complex of Claim 1 wherein the solid
2 support matrix is non-ionizable.

=

5

B

57
--55--

1 13. The resin-protein/peptide complex of Claim 1 wherein the solid
2 support matrix contains ionizable functionality which functionality is
3 electrostatically uncharged at the pH where the target protein or peptide is
4 bound to the resin and is electrostatically charged at the pH where the target
5 protein or peptide is desorbed from the resin.

1 14. The resin-protein/peptide complex of Claim 1 wherein the
2 electrostatic charge induced on the resin of the resin-protein/peptide complex is
3 of the same polarity as the net electrostatic charge on the target protein or
4 peptide at the pH of desorption.

1 15. The resin-protein/peptide complex of Claim 1 wherein the
2 electrostatic charge induced on the resin of the resin-protein/peptide complex is
3 of the opposite polarity from the net electrostatic charge on the target protein or
4 peptide at the pH of desorption.

Handwritten notes: "kgd", "26-13", and other illegible scribbles.

1 16. A resin-protein/peptide complex which comprises a resin and a
2 target protein or peptide bound thereto wherein said resin comprises
3 a) a solid support matrix having a selected ionizable functionality
4 incorporated into the backbone thereof wherein the ionizable functionality is
5 selected such that the resin is electrostatically uncharged at the pH where the
6 target protein or peptide is bound to the resin and is electrostatically charged at
7 the pH where the target protein or peptide is desorbed from the resin; and
8 b) optionally a non-ionizable ligand covalently attached thereto.
9 wherein about 50 percent or more of the target protein or peptide in an
10 aqueous medium binds to the resin when the aqueous medium has either a high
11 or a low ionic strength.

Handwritten notes: "Sub", "P23", "cr", and other illegible scribbles.

1 17. The resin-protein/peptide complex of Claim 16 wherein the
2 ionizable functionality is electrostatically uncharged at the pH where the target

3 protein or peptide is bound to the resin and is positively charged at the pH
4 where the target protein or peptide is desorbed from the resin.

1 18. The resin-protein/peptide complex of Claim 16 wherein the
2 ionizable functionality is electrostatically uncharged at the pH where the target
3 protein or peptide is bound to the resin and is negatively charged at the pH
4 where the target protein or peptide is desorbed from the resin.

1 19. The resin-protein/peptide complex of Claim 16 wherein the
2 ionizable functionality comprises amino groups covalently attached in the
3 backbone of the solid support matrix.

1 20. The resin-protein/peptide complex of Claim 16 wherein the solid
2 support matrix is cross-linked.

1 21. The resin-protein/peptide complex of Claim 16 wherein the resin
2 contains from about 0.05 mmol to about 0.5 mmol non-ionizable ligand per ml
3 of the solid support matrix.

1 22. The resin-protein/peptide complex of Claim 16 wherein the
2 electrostatic charge induced on the resin of the resin-protein/peptide complex is
3 of the same polarity as the net electrostatic charge on the target protein or
4 peptide at the pH of desorption.

1 23. The resin-protein/peptide complex of Claim 16 wherein the
2 electrostatic charge induced on the resin of the resin-protein/peptide complex is
3 of the opposite polarity from the net electrostatic charge on the target protein or
4 peptide at the pH of desorption.

B

59
--57--

1 24. A method for binding and recovering a target protein or peptide
2 from an aqueous medium comprising the target protein or peptide which
3 method comprises:
4 a) contacting the medium with a resin under conditions sufficient to
5 allow the target protein or peptide to bind to the resin wherein said resin
6 comprises a solid support matrix and selected ionizable ligand covalently
7 attached to the matrix wherein the ionizable ligand is selected such that the
8 resin is electrostatically uncharged at the pH where the target protein or peptide
9 is bound to the resin and is electrostatically charged at the pH where the target
10 protein or peptide is desorbed from the resin and further wherein about 50
11 percent or more of the target protein or peptide in an aqueous medium binds to
12 the resin when the aqueous medium has either a high or a low ionic strength;
13 b) separating the resin containing the bound target protein or peptide
14 from the other components of the medium to produce a resin-protein/peptide
15 complex; and
16 c) desorbing the bound target protein or peptide from the complex by
17 contacting the complex with a desorbing solution having a pH which induces an
18 electrostatic charge on the resin wherein the induced charge is of the same
19 polarity as the net charge on the target protein or peptide at the pH of the
20 desorbing solution.

1 25. A method for binding and recovering a target protein or peptide
2 from an aqueous medium comprising the target protein or peptide which
3 method comprises:
4 a) contacting the medium with a resin under conditions sufficient to
5 allow the target protein or peptide to bind to the resin wherein said resin
6 comprises a solid support matrix and selected ionizable ligand covalently
7 attached to the matrix wherein the ionizable ligand is selected such that the
8 resin is electrostatically uncharged at the pH where the target protein or peptide
9 is bound to the resin and is electrostatically charged at the pH where the target
10 protein or peptide is desorbed from the resin and further wherein about 50

11 percent or more of the target protein or peptide in an aqueous medium binds to
12 the resin when the aqueous medium has either a high or a low ionic strength;
13 b) separating the resin containing the bound target protein or peptide
14 from the other components of the medium to produce a resin-protein/peptide
15 complex; and
16 c) desorbing the bound target protein or peptide from the complex by
17 contacting the complex with a desorbing solution having a pH which induces an
18 electrostatic charge on the resin wherein the induced charge is of the opposite
19 polarity from the net charge on the target protein or peptide at the pH of the
20 desorbing solution.

1 26. The method of either Claim 24 or Claim 25 wherein the induced
2 charge on the resin is a positive charge.

1 27. The method of either Claim 24 or Claim 25 wherein the induced
2 charge on the resin is a negative charge.

1 28. The method of either Claim 24 or Claim 25 wherein the aqueous
2 medium is contacted with the resin in a stirred batch process.

1 29. The method of either Claim 24 or Claim 25 wherein the aqueous
2 medium is contacted with the resin in a chromatography column.

1 30. The method of Claim 29 wherein the aqueous medium is contacted
2 with the resin in a fluidized expanded bed.

1 31. The method of Claim 29 wherein the column is a radial flow
2 column.

1 32. The method of either Claim 24 or Claim 25 wherein the aqueous
2 medium is a crude fermentation broth.

6.
61
--59--
1 33. The method of Claim 32 wherein the crude fermentation broth
2 comprises a protein selected from the group consisting of chymosin and
3 subtilisin.

1 34. The method of either Claim 24 or Claim 25 wherein binding of the
2 target protein or peptide to the resin is conducted at a pH of from 2 to 12.

1 35. The method of Claim 34 wherein binding of the target protein or
2 peptide to the resin is conducted at a pH of from 5 to 9.

1 36. The method of Claim 34 wherein desorption of the target protein or
2 peptide from the resin is conducted at a pH within the range of from 2 to 12
3 but at a pH different from that employed to bind the target protein or peptide
4 onto the resin.

1 37. The method of Claim 35 wherein desorption of the target protein or
2 peptide from the resin is conducted at a pH within the range of from 5 to 9 but
3 at a pH different from that employed to bind the target protein or peptide onto
4 the resin.

1 38. The method of Claim 34 wherein the pH of the aqueous mixture is
2 adjusted to from about pH 2 to about pH 12 before contacting the mixture with
3 the resin.

1 39. A method for binding and recovering a target protein or peptide
2 from an aqueous medium comprising the target protein or peptide which
3 method comprises:

4 a) contacting the medium with a resin under conditions sufficient to
5 allow the target protein or peptide to bind to the resin wherein said resin
6 comprises a solid support matrix having a selected ionizable functionality
7 incorporated into the backbone thereof wherein the ionizable functionality is

8 selected such that the resin is electrostatically uncharged at the pH where the
9 target protein or peptide is bound to the resin and is electrostatically charged at
10 the pH where the target protein or peptide is desorbed from the resin wherein
11 about 50 percent or more of the target protein or peptide in an aqueous medium
12 binds to the resin when the aqueous medium has either a high or a low ionic
13 strength;

14 b) separating the resin containing the bound target protein or peptide
15 from the other components of the medium to produce a resin-protein/peptide
16 complex; and

17 c) desorbing the bound target protein or peptide from the complex by
18 contacting the complex with a desorbing solution having a pH which induces an
19 electrostatic charge on the resin wherein the induced charge is of the same
20 polarity as the net charge on the target protein or peptide at the pH of the
21 desorbing solution.

1 40. A method for binding and recovering a target protein or peptide
2 from an aqueous medium comprising the target protein or peptide which
3 method comprises:

4 a) contacting the medium with a resin under conditions sufficient to
5 allow the target protein or peptide to bind to the resin wherein said resin
6 comprises a solid support matrix having a selected ionizable functionality
7 incorporated into the backbone thereof wherein the ionizable functionality is
8 selected such that the resin is electrostatically uncharged at the pH where the
9 target protein or peptide is bound to the resin and is electrostatically charged at
10 the pH where the target protein or peptide is desorbed from the resin wherein
11 greater than 50 percent of the target protein or peptide in an aqueous medium
12 binds to the resin when the aqueous medium has either a high or a low ionic
13 strength;

14 b) separating the resin containing the bound target protein or peptide
15 from the other components of the medium to produce a resin-protein/peptide
16 complex; and

B

63
61

17 c) desorbing the bound target protein or peptide from the complex by
18 contacting the complex with a desorbing solution having a pH which induces an
19 electrostatic charge on the resin wherein the induced charge is of the opposite
20 polarity from the net charge on the target protein or peptide at the pH of the
21 desorbing solution.

1 41. The method of either Claim 39 or Claim 40 wherein the induced
2 charge on the resin is a positive charge.

1 42. The method of either Claim 39 or Claim 40 wherein the induced
2 charge on the resin is a negative charge.

1 43. The method of either Claim 39 or Claim 40 wherein the aqueous
2 medium is contacted with the resin in a stirred batch process.

1 44. The method of either Claim 39 or Claim 40 wherein the aqueous
2 medium is contacted with the resin in a chromatography column.

1 45. The method of Claim 44 wherein the aqueous medium is contacted
2 with the resin in a fluidized bed.

1 46. The method of Claim 44 wherein the column is a radial flow
2 column.

1 47. The method of either Claim 39 or Claim 40 wherein the aqueous
2 medium is a crude fermentation broth.

1 48. The method of Claim 47 wherein the crude fermentation broth
2 comprises a target protein selected from the group consisting of chymosin and
3 subtilisin.

B

64
--62--

1 49. The method of either Claim 39 or Claim 40 wherein binding of the
2 target protein or peptide to the resin is conducted at a pH of from 2 to 12.

1 50. The method of Claim 49 wherein binding of the target protein or
2 peptide to the resin is conducted at a pH of from 5 to 9.

1 51. The method of Claim 49 wherein desorption of the target protein or
2 peptide from the resin is conducted at a pH within the range of from 2 to 12
3 but at a pH different from that employed to bind the target protein or peptide
4 onto the resin.

1 52. The method of Claim 50 wherein desorption of the target protein or
2 peptide from the resin is conducted at a pH within the range of from 5 to 9 but
3 at a pH different from that employed to bind the target protein or peptide onto
4 the resin.

1 53. The method of Claim 49 wherein the pH of the aqueous mixture is
2 adjusted to from about pH 2 to about pH 12 before contacting the mixture with
3 the resin.

1 54. A method for separating a target protein or peptide from an
2 aqueous medium comprising the target protein or peptide which method
3 comprises contacting the medium with a resin under conditions sufficient to
4 allow the target protein or peptide to bind to the resin so as to form a resin-
5 protein/peptide complex as described in either Claim 1 or Claim 16.